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RESEARCH ARTICLE

Genetic and environmental effects on the scaling of metabolic rate with body size

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ABSTRACT

Metabolic rate (MR) often scales with body mass (BM) following a power function of the form $MR=aBM^b$, where log(*a*) is the allometric intercept and *b* is the allometric exponent (i.e. slope on a log–log scale). The variational properties of *b* have been debated, but very few studies have tested for genetic variance in *b*, and none have tested for a genotype-by-environment (G×E) interaction in *b*. Consequently, the short-term evolutionary potentials of both *b* and its phenotypic plasticity remain unknown. Using 10 clones of a population of *Daphnia magna*, we estimated the genetic variance in *b* and assessed whether a G×E interaction affected *b*. We measured MR on juveniles of different sizes reared and measured at three temperatures (17, 22 and 28°C). Overall, *b* decreased with increasing temperature. We found no evidence of genetic variance in *b* at any temperature, and thus no G×E interaction in *b*. However, we found a significant G×E interaction in size-specific MR. Using simulations, we show how this G×E interaction can generate genetic variation in the ontogenetic allometric slope of animals experiencing directional changes in temperature during growth. This suggests that *b* can evolve despite having limited genetic variation at constant temperatures.

KEY WORDS: Allometry, Body size, Constraints, Genotype–environment interaction, Metabolic scaling, Temperature, Evolvability

INTRODUCTION

Life-history, morphological and physiological traits often covary with the size of organisms (Calder, 1984; Schmidt-Nielsen, 1984; Brown and West, 2000). These allometric relationships are typically expressed as a power-law function of the form $Z=aX^b$, where *Z* is the trait value and *X* some measurement of body size (Huxley, 1932). On a log–log scale, the relationship becomes linear: $\log(Z)=\log(a)+b\times\log(X)$, where log(*a*) is the allometric intercept and *b* is the allometric slope (Huxley, 1932; Cock, 1966; Gould, 1966). This slope describes the proportional increase in a trait for a given proportional size increment. Metabolic rate (MR) is one of the phenotypic traits that has received much attention with respect to its allometric relationship with body mass (BM). For this trait, a common assertion has been that the allometric slope, *b*, is invariant, with a value of approximately 3/4 (i.e. the '3/4-power law') (Kleiber, 1961; Calder, 1984; Brown and West, 2000). Furthermore, this apparent constancy has led to the suggestion that the allometric relationship between MR and BM may constrain other allometric relationships (e.g. West et al., 1997) because metabolism entails all transformations of materials and energy into various life structures and functions (Calder, 1984; Schmidt-Nielsen, 1984; Brown and West, 2000; Glazier, 2005). These observations have also led to the development of theories such as the 'resource-transport network theory' (West et al., 1997), which aims at explaining why *b* should equal 3/4, and the 'metabolic theory of ecology', which uses the model by West et al. (1997) in an attempt to explain scaling of various ecological processes (Brown et al., 2004; Sibly et al., 2012).

Although the 3/4-power law has traditionally been considered a good approximation for the metabolic allometry of some taxonomic groups (e.g. mammals; Kleiber, 1961), critics emphasize that there is considerable interspecific variation in the value of *b* (see Glazier, 2005, 2010 and references therein), including for mammals (e.g. White and Seymour, 2003). Glazier (2010) hypothesized that this variation was generated by the overall level of metabolic activity of the organism (i.e. the metabolic-level boundaries hypothesis). Accordingly, MR should scale proportionally to BM (i.e. $b=1$) in organisms with low activity, because these organisms are not limited by fluxes of resources, waste or heat. In contrast, for organisms with high activity and a metabolism that is constrained by the surface area available for such fluxes, *b* should be close to 2/3 following surface/volume ratio arguments (i.e. $\text{surface}\propto\text{volume}^{2/3}$, causing larger individuals to have a smaller surface area per unit volume). By extension, one would predict that genetic variance in overall activity within species would translate into genetic variance in *b*, and hence that *b* can evolve. Whereas studies demonstrating genetic variance in the allometric intercept of MR are becoming increasingly common (e.g. Sadowska et al., 2005; Yashchenko et al., 2016; Pettersen et al., 2018), empirical studies testing for within-population genetic variation in the value of *b* are largely lacking, preventing us to assess its evolutionary potential. Although interspecific variation in *b* suggests that *b* has evolved over long time scales in concert with evolutionary changes in body plans, behavior and physiology, knowledge about the evolutionary potential is critical to enable predictions on shorter time scales.

Ambient temperature is a major source of variation in *b* (Glazier, 2005). While it is well known that MR normally increases exponentially with temperature for a given body size (within natural temperature ranges), the effect of temperature on *b* varies considerably, with studies showing no effect, a linear positive or negative effect, or a humped relationship (Glazier, 2005). However, genetic variation in the thermal reaction norm of *b* [i.e. a genotype-by-environment (G×E) interaction] has never been assessed. Considering the predicted changes in global temperature patterns, knowing the evolutionary potential of the thermal plasticity of *b* is crucial for predicting the ability of populations to adapt to these changes. For example, a population may have evolved thermal plasticity in *b* that optimizes *b* under commonly experienced temperatures. This may, however, come at the cost of having a suboptimal *b* at less experienced temperatures.

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If temperature regimes change, this population will need to evolve a new plastic response that optimizes *b* in the new environment.

In this study, we used the crustacean zooplankton *Daphnia magna* to assess genetic variance in both intercepts and slopes of MR allometries. By performing the experiment at three different temperatures (17, 22 and 28°C), we also assessed G×E interactions on the allometric intercept and slope. We measured MR of juveniles originating from 10 different genotypes from a single population, and with sizes varying over an order of magnitude within each genotype. We estimated the allometric relationship for each genotype within each environment, the overall genetic variance in the allometric intercept and slope, and tested for G×E interactions on the allometric intercept and slope.

MATERIALS AND METHODS

Study animals and husbandry

Ephippia of *Daphnia magna* Straus 1820, which contain eggs that are the product of sexual reproduction, were collected in November 2014 from the surface sediment of a pond at Værøy Island (Sandtjønna, 1.0 ha, 67.687°N 12.672°E), northern Norway. Ten genotypes, hereafter referred to as clones, were hatched from separate ephippia in December 2014 and cultured separately under common garden conditions for 18–22 months (35–50 asexual generations). Being a result of sexual reproduction, each clone is genetically unique at the molecular level. Although molecular genetic variation among these clones is unknown, previous quantitative genetic experiments on thermal plasticity of life-history traits performed on the same clones have demonstrated that these clones harbor substantial quantitative genetic variation (Fossen et al., 2018). Notice that quantitative genetic measures are recommended over indirect molecular measures when evaluating the short-term evolutionary potential of populations (Reed and Frankham, 2001). Animals were kept in 250 ml jars containing a modified ADaM medium (Klüttgen et al., 1994; SeO_2 concentration reduced by 50%, sea-salt increased to 1.23 g l^{-1}) at 17°C with a 16 h:8 h light:dark photoperiod. Cultures containing five adults per jar were fed three times a week with Shellfish Diet 1800 (Reed Mariculture Inc., USA) at a final algae concentration of 4×10^5 cells ml^{-1}. The medium was changed weekly.

Measurement of metabolic rate and body size

MR was measured as oxygen consumption of fed, free-swimming individuals at three different temperatures (17, 22 and 28°C) following the method described in Yashchenko et al. (2016). This measure of MR measures the total MR during normal activity, and varies very little over time during measurements (Yashchenko et al., 2016). Oxygen consumption was measured in a sealed glass microplate equipped with planar oxygen sensor spots with optical isolation glued onto the bottom of 200 µl wells (Loligo Systems, Denmark) integrated with a 24-channel fluorescence-based respirometry system (SDR SensorDish® Reader, PreSens, Germany). *Daphnia* were transferred into wells with air-saturated ADaM, which were sealed using an adhesive PCR film (Thermo Scientific, Waltham, MA, USA) while ensuring no air bubbles in the wells. The reader was placed inside a Memmert Peltier-cooled incubator IPP (Memmert, Germany) at a constant experimental temperature, which was also used to temperate the equipment and ADaM prior to measurements. Preparation of the plate was conducted in a steel container placed in a water bath with the same experimental temperature. Oxygen concentrations inside wells were measured in darkness every 3 min for a duration of 120–150 min by using SDR v38 software (PreSens, Germany). Wells with medium but without animals were used to control for temporal changes in pressure and temperature. Oxygen consumption was estimated from the decline in oxygen concentration during the interval of time where this decline is linear after controlling for oxygen diffusion into the wells. The gut length (GL; in mm; measured from the top of midgut to the bottom of hindgut when the animal is relaxed) of the animals was measured (ImageJ v1.48, National Institutes of Health, Bethesda, MD, USA) from photographs taken under a stereomicroscope immediately after respiration measurements. Dry mass (DM; in mg) was estimated as $DM=0.00679GL^{2.75}$ (Fossen et al., 2018).

Experimental design

Female juveniles were sampled from the second or later clutches of stock-culture animals maintained at 17°C, and moved into Memmert Peltier-cooled incubator IPP 260plus (Memmert, Germany) climate cabinets set at 17, 22 and 28°C. These temperatures had relatively low and similar juvenile mortality (Fossen et al., 2018). The animals were reared at these temperatures for a minimum of three asexual generations. Each generation was started from juveniles from the second or later clutches born in different jars to obtain independent replicates of clones. Rearing conditions were similar to conditions in stock cultures, but animals were fed with temperature-specific amounts of food every second day (2.62×10^5 cells ml^{-1} at 17°C; 3.24×10^5 cells ml^{-1} at 22°C; 4.00×10^5 cells ml^{-1} at 28°C), and the medium was changed every 6 days at 17°C, every 4 days at 22°C and every 2 days at 28°C. The food supply represents *ad libitum* concentrations. The position of jars containing the different clones within a cabinet was rotated after every medium change.

Female juveniles from second or later clutches and from independent jars were used for the measurements. To obtain a large range of sizes and estimate the allometric relationship with high precision, each experimental run consisted of one large [close to maturity, mean (s.d.) dry mass: 0.053 (0.012) mg] and one small [recently born, mean dry mass: 0.007 (0.004) mg] juvenile from each of the 10 clones. By using juveniles we ensured that all animals were in the same non-reproductive physiological and metabolic state (Glazier, 1991; Gaitán-Espitia et al., 2013). Four out of the 24 wells of the plate were left empty (wells with medium but without animals) to be used as controls. In total, 13–15 runs were conducted per temperature, resulting in 23–30 individuals per clone per temperature and a total sample size of 798 measurements (Table S1). The experiment was conducted over 6 months in 2016 (May–November) where, on any given day, at most two experimental runs were conducted. Two different microplates were used alternately throughout the experiment. Animals were last fed 1 or 2 days before respiration measurements (independent of temperature), but all animals within a run were fed at the same time.

Statistical analyses

All statistical analyses were conducted in R v.3.1.1 (http://www.R-project.org/). We fitted linear mixed-effect models using the package lme4 (v. 1.1-7; Bates et al., 2015) to estimate the genetic variance in the intercepts and slopes of the allometric relationship between MR and BM at different temperatures. Such mixed-effect models have been shown to give more accurate estimates of variances than alternative two-step approaches that first estimate intercepts and slopes for each genotype (Morrissey and Liefting, 2016). Log_e-transformed oxygen consumption (mg h^{-1}) was the response variable. Predictor variables were log_e dry mass (BM; mg), temperature (*T*; categorical), a T×BM interaction, plate ID (categorical: plate I and plate II), last feeding time (FT; categorical,

two levels: 1 or 2 days before experiment), and interactions between feeding time, dry mass and temperature (FT×BM, FT×T and FT×BM×T interactions). Run ID (categorical, 42 levels), well ID (categorical, 40 levels; 20 wells×2 plates) and clone (categorical) were random effects. Run ID and well ID were assumed to only affect the overall intercept. Clone could affect temperature-specific intercepts and temperature-specific slopes (i.e. testing for a G×E interaction in intercept or slopes, respectively). The estimated variance due to clone represents the total (broad sense) genetic variance in allometric parameters (i.e. temperature-specific intercepts and slopes). We obtained 95% confidence intervals of these variance components using 1000 bootstrap simulations on a model that included all random effects. Model selection was done using Akaike information criterion corrected for small sample sizes (AICc). First, we compared models with different random effect using the full model fitted with restricted maximum likelihood (REML). Then we compared models with different fixed effects with maximum likelihood (ML) using the random effect structure from the best fitted model. Lastly, we obtained parameter estimates from the best model fitted with REML. Pseudo-R^2 values were calculated as the squared correlation coefficient between predicted values from the model and observed values.

To illustrate the effect of temperature on MR and test whether this effect is size dependent, we first estimated oxygen consumption at two different sizes (dry mass of small group: $e^{-5.0}$=0.0066 mg; large group: $e^{-3.0}$=0.052 mg). We fitted the best model describing the effect of size, temperature and their interaction on MR, but without clone as a random effect. We then obtained size- and temperature-specific measures of oxygen consumption by adding to the residuals of this model the expected O_2 consumption for the two size groups at each temperature. For both measurements of size-standardized oxygen consumption, an overall temperature effect was calculated as:

$$Q_{10} = \left(\frac{\mathrm{MR}_2}{\mathrm{MR}_1}\right)\left(\frac{10}{T_2 - T_1}\right), \quad (1)$$

where MR_1 and MR_2 are the oxygen consumptions (mg h^{-1}) at two different temperatures (T_1 and T_2, respectively). Q_{10} is a dimensionless measure that represents the proportional increase in oxygen consumption with a temperature increase of 10°C. It was calculated for all three combinations of temperatures (T=17 and 22°C; T=17 and 28°C; T=22 and 28°C). Using those size-standardized MRs, we quantified the thermal reaction norm of MR at each size (small and large) and estimated the genotype-by-temperature interaction by using clone as a random effect. We fitted quadratic mixed-effect models with $\log_e$ size-standardized oxygen consumption as the response variable, temperature and temperature^2 as covariates and clone as the random effect. Clone could affect the intercept, the slope and the curvature (i.e. quadratic term) in the model. Model selection was conducted using AICc with the same procedure as described above.

Estimating evolutionary potential

The population's evolutionary potential of MR was estimated as broad sense evolvability (clonal variance/mean^2) within temperatures (Houle, 1992; Hansen et al., 2003, 2011). Evolvability measures the expected percentage change in a trait per generation under a unit strength of selection. Compared to heritability, evolvability is independent from the environmental variance and represents a measure of the evolutionary potential that is comparable across traits, populations and species (Hansen et al., 2011). Given that there is no genetic variance in the allometric slope, the clonal variance in allometric intercept is equal to broad sense evolvability because MR is estimated on a natural-logarithmic scale.

RESULTS

Overall, the allometric slope of MR decreased with temperature and each temperature-specific slope was statistically significantly different from 3/4 (Table 1, Fig. 1). We found no evidence of genetic variance in the allometric slopes at any temperature, as indicated by the structure of the random effect of the best models (Table 1, Table S2). Consequently, there was also no G×E interaction in the allometric slopes. However, we found evidence of genetic variance in the allometric intercepts within temperatures (Table 1, Table S2). Evolvability of the allometric intercept was similar at 17 and 22°C (0.187 and 0.157%, respectively), but higher at 28°C (0.556%, Table 1). For a given size, the MR was 13, 12 and 25% higher for the clone with the highest metabolism compared with the clone with the lowest metabolism at 17, 22 and 28°C, respectively. There was no evidence for an effect of plate or feeding time or interaction effects of feeding time and BM and/or temperature (Table S2).

Metabolism increased with temperature. The reaction norm was non-linear and displayed clonal variance in the intercept, slope and quadratic term: 7.1×10^{-4}, 2.1×10^{-5} and 2.8×10^{-6}, respectively, and the residual variance was 2.8×10^{-2} [$\log_e$(mg O_2 h^{-1})]^2. However, the strength and shape of the increase of metabolism with temperature depended on the size of the animals. The effect of increasing temperature on MR represented by Q_{10} was larger for smaller individuals than for larger ones (Table 2). Furthermore, small animals tended to increase their metabolism linearly with temperature, whereas the increase was non-linear for larger individuals and more pronounced between 22 and 28°C than between 17 and 22°C (Fig. 2, Table 2). Finally, we found clear evidence for differences among clones in the curvature of the reaction norm for both small and large individuals (Fig. 2, Table S3).

DISCUSSION

We quantified genetic variation in the allometric relationship between MR and BM at different temperatures. Allometric slopes were generally steeper than 3/4 and became shallower with increasing temperature. Within temperatures, we found evidence

Table 1. Allometric intercepts, slopes and variance components for log metabolic rate (MR) of 10 clones from a population of *Daphnia magna*

Temperature (°C)	Intercept (±s.e.)	Slope (±s.e.)	$V_{clone,intercept}$ (95% CI)	$V_{clone,slope}$ (95% CI)
17	−4.62±0.05	0.944±0.010	1.87×10^{-3} (0.10×10^{-3}, 5.06×10^{-3})	3.50×10^{-16} (0, 1.78×10^{-4})
22	−4.66±0.06	0.860±0.010	1.57×10^{-3} (0.13×10^{-3}, 4.92×10^{-3})	1.78×10^{-15} (0, 1.62×10^{-4})
28	−4.32±0.06	0.834±0.010	5.56×10^{-3} (1.23×10^{-3}, 13.36×10^{-3})	4.64×10^{-16} (0, 3.77×10^{-4})

Estimates are from a mixed-effect model with clone as a random effect. Pseudo-R^2=0.97. $V_{run}=1.52\times10^{-2}$, $V_{well}=6.82\times10^{-3}$, $V_{res}=3.00\times10^{-2}$. $V_{clone,intercept}$ and $V_{clone,slopes}$, clonal variance in intercept and slope, with 95% confidence intervals (CI) obtained by bootstrapping (1000 simulations); V_{run} and V_{well}, variance in intercepts because of differences among runs and wells; V_{res}, overall residual variance. The 95% CI of the population slopes (slope±1.96 s.e.) do not include 3/4. Note that the best model included temperature-specific clonal variance in intercepts, but not in slopes. MR was measured in mg oxygen consumed h^{-1}.

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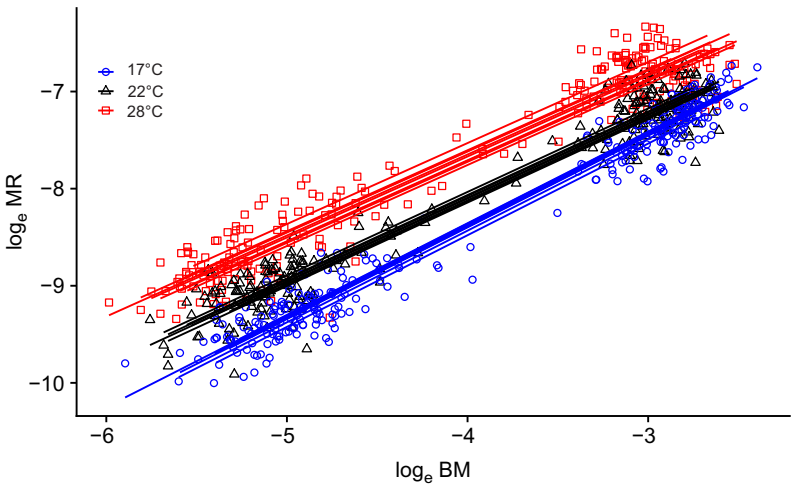

Fig. 1. Allometric relationship of metabolic rate (MR) for 10 clones of a population of *Daphnia magna* at three different temperatures. Each line represents one clone, the same clones being used at each temperature. Lines are fitted from best linear unbiased predictions of the random effects from a mixed-effect model. Within temperatures, clones have different intercepts, but the same slopes. See Table S4 for clone specific intercepts. MR was measured in mg oxygen consumed h^{-1}, and body mass (BM) in mg.

for genetic variation in the allometric intercepts, but not in the allometric slopes. Consequently, there was also no genetic variance in how the slope changes with temperature (i.e. no G×E interaction in allometric slopes). The observed broad sense evolvability of the allometric intercept (0.19–0.56%) is similar to evolvabilities observed for physiological traits (median evolvability: 0.49%; Hansen et al., 2011). Additionally, we found genetic variance in how size-specific MR changed with temperature (i.e. a G×E interaction). Both these results support other studies that have found significant genetic variance in MR after controlling for body size (e.g. Bouchard et al., 1989; Dohm et al., 2001; Yashchenko et al., 2016; Pettersen et al., 2018), or have found a G×E interaction in the allometric intercept (e.g. Arnqvist et al., 2010; Niitepold, 2010). This suggests that thermal reaction norms of size-specific MRs have the potential to evolve. In contrast, the lack of genetic variance in the allometric slope of MR and how this slope changes with temperature suggests that this slope and its thermal plasticity have a limited potential for evolving over short time scales (Hansen, 2015). This would be expected if the population from which these clones originated had overall low genetic variation. However, both the presence of a G×E interaction in the allometric intercept (Fig. 2) and the genetic variance observed in thermal plasticity of life-history traits among the same clones (Fossen et al., 2018) suggest that this population harbors sufficient genetic variation to be detected with 10 clones for traits displaying normal levels of evolvability (Hansen et al., 2011). Although more studies are needed to know how general these results are, they do support the lack of differences in allometric slope observed among smaller sets of *Daphnia* clones in two previous studies (Glazier and Calow, 1992; Yashchenko et al., 2016). The allometric slope's apparent lack of evolvability may be explained by pleiotropic constraints, where correlated developmental traits are strongly negatively affected by changes in metabolic allometry (Riedl, 1977; Bolstad et al., 2015). MR strongly affects the development of other traits (e.g. Schmidt-Nielsen, 1984; Brown et al., 2004). If this lack of genetic variance is a general finding, it may explain why allometric slopes of other traits that may be functionally linked to MR often appear genetically constrained (e.g. morphological traits; Pélabon et al., 2014; Voje et al., 2014).

In contrast to the lack of genetic variance in allometric slopes, we did observe phenotypic plasticity in metabolic scaling, as the allometric slope decreased with increasing temperature. This

Table 2. Effects of temperature on MR in 10 clones from a population of *D. magna*

Size	Temperature combination (°C)	Q_{10} (95% CI)
Small	17–22	2.1 (1.9, 2.4)
	17–28	2.2 (2.1, 2.3)
	22–28	2.2 (2.1, 2.4)
Large	17–22	1.5 (1.4, 1.7)
	17–28	1.8 (1.7, 1.9)
	22–28	2.0 (1.9, 2.1)

Q_{10} was calculated separately for MR standardized to a large (ln BM=−3) and small (ln BM=−5) body size, and when using different combinations of temperature. MR was measured in mg oxygen consumed h^{-1}.

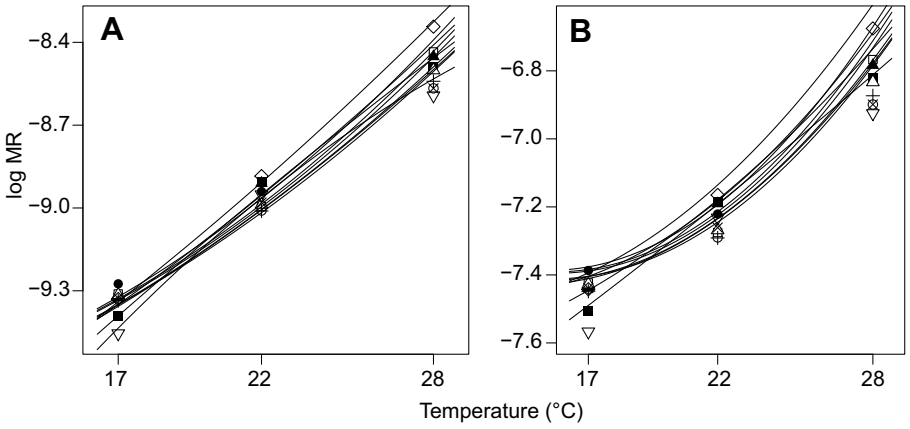

Fig. 2. Genotype-by-environment (G×E) interaction in thermal reaction norms of MR for 10 clones of a population of *D. magna* at two different sizes. (A) Reaction norm for small (ln BM=−5) juveniles. Regression line for the whole population (±s.e.): ln(MR)=−8.96 (±0.01)+0.077 (±0.002)×T+0.0001 (±0.0006)×T^2, where T=temperature centered at 22°C. Pseudo-R^2=0.82. (B) Reaction norm for large (ln BM=−3) juveniles. Regression line for the whole population (±s.e.m.): ln(MR)=−7.24 (±0.01)+0.055 (±0.002)×T+0.0024 (±0.0006)×T^2. Pseudo-R^2=0.72. Note the difference in y-scale. See Table S5 for clone-specific reaction norm parameters. MR was measured in mg oxygen consumed h^{-1}.

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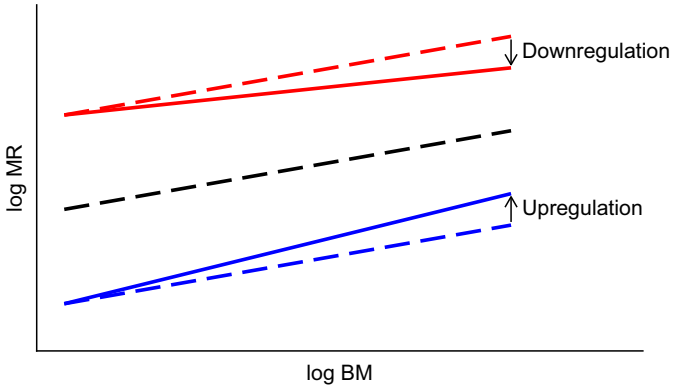

Fig. 3. The acclimation hypothesis for temperature dependence in body size scaling of MR. Red lines represent high temperature, black lines intermediate temperature and blue lines low temperature. Ectotherms reared at low or high temperatures up or downregulate their metabolism, respectively (Kielland et al., 2017), probably as a means to counteract direct temperature effects that suboptimally reduce or increase their metabolism. If there are no such acclimation processes or these are completed at birth, the allometric slopes should be parallel across temperatures (dashed lines). However, if acclimation occurs post-birth, animals at low temperatures will upregulate their metabolism throughout ontogeny. This will result in steeper allometric slopes (solid blue line). Similarly, for high temperatures, animals will downregulate metabolism throughout ontogeny and end up with flatter allometric slopes (solid red line).

negative relationship between metabolic scaling and temperature is not uncommon for ectotherms (e.g. Barlow, 1961; Barnes and Barnes, 1969; Xie and Sun, 1990; Carey and Sigwart, 2014), but other relationships have also been found (Glazier, 2005). Negative relationships between the slope (b) and temperature have been predicted for ectotherms by the metabolic-level boundaries hypothesis (MLBH; Glazier, 2010). According to this hypothesis, as temperature increases, the energetic costs of routine metabolism also increases, thus increasing the importance of surface-area constraints at larger body sizes (Glazier, 2010; Killen et al., 2010). Alternatively, Verberk and Atkinson (2013) suggested that MR decreases at lower temperatures because the higher water viscosity of cold water increases the thickness of boundary layers enveloping respiratory surfaces. This effect should be stronger in small individuals, which are more sensitive to the increased viscosity and have more difficulty ventilating at low temperatures (Verberk and Atkinson, 2013). Both hypotheses are based on constraint arguments, and predict a convergence of MRs across temperatures with increasing BM, as observed in the current study (Fig. 1). However, we suggest an alternative acclimation hypothesis, whereby organisms attempt to reduce the direct effect of temperature by up- or downregulating MRs when growing in cold or warm environments, respectively (Fig. 3). At least for *Daphnia*, such regulatory responses of metabolism to rearing temperature are observed to occur throughout the juvenile stage (Kielland et al., 2017). Unless animals have completed their acclimation at birth (due to strong maternal effects and/or acclimation during the embryonic stage), this acclimation is expected to occur throughout ontogeny even if animals are born and reared at a constant temperature for multiple generations, as was the case with our animals. This would cause a similar convergence of MRs across temperatures for older and larger individuals, which are better acclimated than newly born ones. Specific experiments are necessary to distinguish between these three alternatives for ours and other similar study systems. However, the MLBH may be more generally applicable as it can also explain cases where b varies with regards to other factors (e.g. Killen et al., 2010), and cases where acclimation is not involved (e.g. Carey and Sigwart, 2014).

Most of our results contradict predictions made by the metabolic theory of ecology (MTE; Brown et al., 2004). Firstly, the occurrence of phenotypic plasticity in metabolic scaling contradicts the assumption of an independent influence of body size and

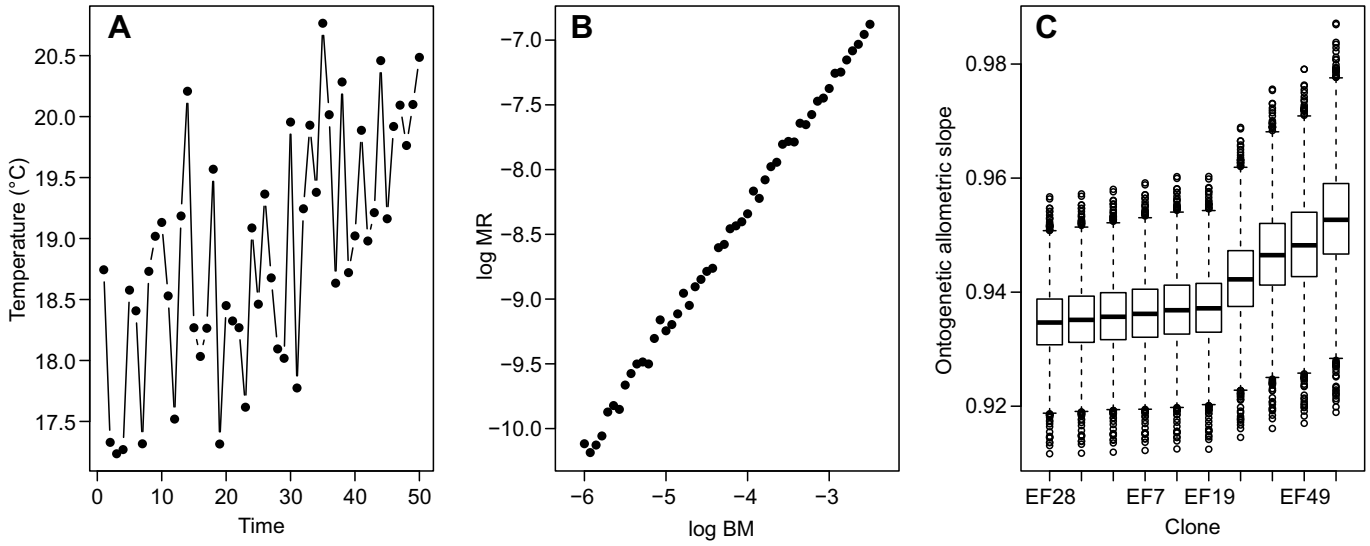

Fig. 4. Simulations of how a G×E interaction in the reaction norm between size-specific MR and temperature can generate genetic variation in the ontogenetic allometric slope between body size and MR. A total of 5000 simulations were run, each consisting of 50 time steps. Log BM increased linearly with time over the same size range as the empirical data. For each run, temperature was drawn from a normal distribution with s.d.=0.75, where the mean temperature increased linearly over time from 18 to 20°C (A). For each time step, clone-specific reaction norm parameters from Fig. 2 were used to predict the MR of each of the 10 clones at the given body size and temperature. (B) Illustration of the corresponding predicted oxygen consumption for one of the clones given its BM and experienced temperature. (C) Box- and whisker-plot showing simulated ontogenetic allometric slopes between BM and MR obtained for each clone. Black bands represent the median, top and bottom of boxes represent the first and third quartiles, and the whiskers the maximum and minimum values when excluding outliers. Based on these simulations, the mean allometric slope observed across ontogeny was 0.941 and the heritability 0.44. MR was measured in mg oxygen consumed h^{-1}, and BM in mg.

temperature on MR (Killen et al., 2010; Glazier, 2015). Accounting for this plasticity is likely to be important for understanding how populations and communities can respond to climate change (Lindmark et al., 2018). In particular, such effects must be accounted for when predicting age- and size-specific ecological responses to temperature. Secondly, the allometric slopes that we observed were higher (b=0.83 to 0.94) than the predicted b=3/4, thus supporting previous observations on *Daphnia* (Glazier, 1991) and other pelagic invertebrates (Glazier, 2006). Thirdly, MTE predicts a relatively constant temperature response of Q_{10}≈2.5 (equivalent to an 'activation energy' of E≈0.65) for all body sizes. In contrast, we observed a temperature response that depended both on the size of the individuals (larger increase for small individuals) and the temperature range considered (larger increase between 22 and 28°C). This finding is consistent with other studies that showed variation in response to temperature depending on the size of individuals or the temperature range (e.g. Armitage, 1962; Cech et al., 1994; Downs et al., 2008; Glazier, 2015). Lastly, not only does our observed Q_{10} vary, but the value of Q_{10} for the largest individuals was considerably lower (Q_{10}=1.5 to 2.0, equivalent to E=0.37 to 0.45) than the value predicted by MTE.

Conventional arguments suggest that the lack of genetic variance in the allometric slope should strongly constrain its evolutionary rate (*sensu* Hansen, 2015). However, the observed G×E interaction between size-specific MR and temperature may cause genetic variation in the ontogenetic allometry between BM and metabolism for populations in the wild. Indeed, for individuals experiencing directional changes in temperature during ontogeny, the ontogenetic allometric slope between BM and MR will vary across genotypes. To illustrate this point, we ran simulations where the temperature fluctuated around an increasing mean temperature (from 18 to 20°C) during ontogeny and where the clone-specific parameters were used to calculate the allometric slope between MR and BM (Fig. 4). This fluctuation mimics how temperature increases towards summer. Our simulation shows that these directional changes in temperature generate consistent differences among clones in the ontogenetic allometric slope. This is due to clones responding differently to changes in temperature during ontogeny, and because BM and temperature covary during this period. Although the simulated differences in allometric slope among clones are small (range in slopes: 0.013), this among-clone difference depends on the temperature regime used in the simulations. For example, a more extreme but still realistic scenario where mean temperature increases from 15 to 20°C during ontogeny yields a four-times larger genetic variance in the slopes (range in slopes: 0.048). Although these slopes result from variation in both BM and environment, and hence are not strictly allometries, this process may allow ontogenetic allometry between MR and BM to evolve despite the absence of genetic variation in allometric slopes at constant temperature. This type of genetic variation in slopes could be of particular relevance for evolution under situations where there are consistent temporal correlations between temperature and some other environmental factor that influence the optimal MR (e.g. food abundance; Einum, 2014), causing selection for different metabolisms at different temperatures.

In this study, we quantified the effects of temperature and quantitative genetics on metabolic scaling slopes. Despite using an experimental design maximizing the power to detect variation in these allometric slopes, with large variation in BM and highly precise measurements of genetically variable clones, we found no evidence for genetic variance or G×E interactions in the allometric slope between BM and MR. However, we showed that this lack of genetic variance in the allometric slope can be compensated by a G×E interaction in the allometric intercept of size-specific MR when the environment changes during ontogeny. We demonstrated such an effect using temperature as the environmental variable, but this should also apply to any other environmental factor that undergoes directional changes throughout the growing season (e.g. food abundance or photoperiod), and show G×E interactions in the allometric intercept of size-specific MR. These results suggest that a better understanding of the complex interplay between the environment and body size is needed to make better and more realistic models to predict and explain variation in metabolic scaling.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: E.I.F.F., C.P., S.E.; Methodology: E.I.F.F., C.P., S.E.; Formal analysis: E.I.F.F., C.P., S.E.; Investigation: E.I.F.F.; Writing - original draft: E.I.F.F.; Writing - review & editing: E.I.F.F., C.P., S.E.; Visualization: E.I.F.F., C.P., S.E.; Supervision: C.P., S.E.; Funding acquisition: S.E.

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Data availability
Data are available from the Dryad Digital Repository (Fossen et al., 2019): https://doi.org/10.5061/dryad.c538kq7.

Supplementary information
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Supporting Information

Table S1. Sample sizes per clone by temperature combination used in metabolic rate experiment for 10 clones from a population of *Daphnia magna*.

	EF19	EF28	EF49	EF50	EF58	EF63	EF64	EF7	EF86	EF88
17°C	29	28	27	30	29	30	29	29	27	30
22°C	26	28	25	27	25	25	28	25	23	24
28°C	25	26	25	26	24	26	26	25	26	25

Table S2. Model selection using AICc of candidate models for metabolic rate allometry of 10 clones from a population of *Daphnia magna*. Models sorted by Δ AICc. The best random effect structure was first determined with REML on models that included all listed fixed effects. Fixed effects were then compared with ML using the best random effect structure. MR = oxygen consumption (mg h^{-1}), BM = body mass (mg), FT = last feeding time, T = temperature, K = number of parameters. The least complex model within 2 Δ AICc is bolded.

	Model	K	AICc	Δ AICc	Akaike weights
Fixed effects	ln MR ~ ln BM + T + ln BM:T + FT + plate	17	-319.42	0.00	0.26
	ln MR ~ ln BM + T + ln BM:T + FT + FT:T + plate	19	-319.07	0.34	0.22
	ln MR ~ ln BM + T + ln BM:T	15	-318.17	1.24	0.14
	ln MR ~ ln BM + T + ln BM:T + FT + FT:BM + plate	18	-318.10	1.31	0.13
	ln MR ~ ln BM + T + ln BM:T + FT + FT:BM + FT:T + plate	20	-317.76	1.66	0.11
	ln MR ~ ln BM + T + ln BM:T + plate	16	-317.70	1.72	0.11
	ln MR ~ ln BM + T + ln BM:T + FT + FT:BM + FT:T + FT:MB:T + plate	22	-314.64	4.78	0.02
	ln MR ~ ln BM + T	13	-259.41	60.01	0.00
	ln MR ~ ln BM	11	-214.41	105.01	0.00
	ln MR ~ T	12	2240.96	2560.38	0.00
Random effects	**(T | clone) + (1 | run) + (1 | well ID)**	22	-245.69	0.00	0.70
	(T | clone) + (ln BM | clone) + (1 | run) + (1 | well ID)	23	-243.57	2.12	0.24
	(1 | clone) + (1 | run) + (1 | well ID)	17	-240.65	5.04	0.06
	(T | clone) + (T : ln BM | clone) + (1 | run) + (1 | well ID)	28	-232.89	12.81	0.00
	(1 | run) + (1 | well ID)	16	-221.53	24.16	0.00
	(T | clone) + (T : ln BM | clone) + (1 | run)	27	-185.14	60.55	0.00
	(T | clone) + (T : ln BM | clone) + (1 | well ID)	27	-62.75	182.94	0.00

Table S3. Model selection using AICc of candidate models for the effect of temperature on metabolic rate of 10 clones from a population of *Daphnia magna*. Metabolic rate was analyzed separately when standardized to a small versus a large body size. Models sorted by Δ AICc. The best random effect structure was first determined with REML on models that included all listed fixed effects. Fixed effects were then compared with ML using the best random effect structure. MR = oxygen consumption (mg h^{-1}), T = temperature, K = number of parameters. The best model is bolded.

Body size		Model	K	AICc	Δ AICc	Akaike weights
Small	Fixed effects	**ln MR ~ T**	6	-553.29	0.00	0.73
		ln MR ~ T + T^2	7	-551.32	1.97	0.27
		ln MR ~ 1	5	-503.97	49.32	0.00
	Random effects	**(1 | clone) + (T | clone) + (T^2 | clone)**	7	-520.55	0.00	0.76
		(1 | clone) + (T | clone)	6	-517.61	2.94	0.18
		(1 | clone)	5	-515.56	4.99	0.06
Large	Fixed effects	**ln MR ~ T + T^2**	7	-551.32	0.00	0.99
		ln MR ~ T	6	-542.32	9.00	0.01
		ln MR ~ 1	5	-499.51	51.81	0.00
	Random effects	**(1 | clone) + (T | clone) + (T^2 | clone)**	7	-520.55	0.00	0.76
		(1 | clone) + (T | clone)	6	-517.61	2.94	0.18
		(1 | clone)	5	-515.56	4.99	0.06

Table S4. Predicted and estimated parameters for metabolic rate allometry of 10 clones from a population of *Daphnia magna*. Two models were used: one mixed-effect model with clone as a random effect, ln oxygen consumption (mg h^{-1}) as the response and ln body mass (mg) as a covariate (full model given in Table S2), and one with clone as a fixed effect. For the model with clone as a random effect, parameters were fitted from BLUPs, and only intercepts are given for each clone since slopes were found to not differ significantly (Table S2). Within temperatures, clones have been sorted in descending order of the intercept BLUPs. Note that the order of clones differ somewhat between models due to the model with clones as a fixed effect not properly accounting for other random effects.

Temperature (°C)	Clone	Intercept from model with clone as random effect	Intercept (± SE) from model with clone as fixed effect	Slope (± SE) from model with clone as fixed effect
17	EF19	-4.585	-4.499 ± 0.067	0.993 ± 0.031
	EF50	-4.585	-4.536 ± 0.067	0.938 ± 0.029
	EF86	-4.600	-4.559 ± 0.067	0.921 ± 0.035
	EF63	-4.602	-4.539 ± 0.066	0.934 ± 0.031
	EF58	-4.614	-4.548 ± 0.068	0.924 ± 0.030
	EF28	-4.617	-4.551 ± 0.067	0.988 ± 0.032
	EF64	-4.617	-4.562 ± 0.066	0.946 ± 0.032
	EF7	-4.623	-4.549 ± 0.067	0.965 ± 0.032
	EF49	-4.657	-4.620 ± 0.068	0.914 ± 0.031
	EF88	-4.704	-4.689 ± 0.066	0.918 ± 0.034
22	EF86	-4.595	-4.528 ± 0.071	0.869 ± 0.035
	EF49	-4.627	-4.540 ± 0.070	0.865 ± 0.033
	EF58	-4.636	-4.549 ± 0.070	0.879 ± 0.032
	EF19	-4.656	-4.583 ± 0.069	0.857 ± 0.032
	EF88	-4.660	-4.595 ± 0.070	0.846 ± 0.033
	EF50	-4.661	-4.634 ± 0.069	0.872 ± 0.035
	EF63	-4.681	-4.638 ± 0.070	0.842 ± 0.035
	EF7	-4.693	-4.623 ± 0.069	0.828 ± 0.035
	EF64	-4.694	-4.656 ± 0.069	0.893 ± 0.034
	EF28	-4.704	-4.661 ± 0.070	0.849 ± 0.030
28	EF86	-4.195	-4.110 ± 0.071	0.855 ± 0.032
	EF19	-4.269	-4.219 ± 0.071	0.834 ± 0.035
	EF58	-4.277	-4.227 ± 0.072	0.867 ± 0.031
	EF50	-4.282	-4.202 ± 0.070	0.862 ± 0.031
	EF49	-4.313	-4.259 ± 0.071	0.848 ± 0.033
	EF63	-4.334	-4.280 ± 0.070	0.841 ± 0.032
	EF64	-4.372	-4.320 ± 0.070	0.782 ± 0.032
	EF7	-4.378	-4.346 ± 0.071	0.790 ± 0.034
	EF28	-4.388	-4.345 ± 0.069	0.822 ± 0.035
	EF88	-4.421	-4.377 ± 0.071	0.835 ± 0.031

Table S5. Predicted parameters for the effect of temperature on metabolic rate of 10 clones from a population of *Daphnia magna* at two different sizes. The parameters are fitted from BLUPs of the random effects from a mixed-effect model with ln oxygen consumption (mg h^{-1}) as the response. Clones differed significantly in the elevation and curvature of the reaction norms.

Size	Clone	Intercept	Slope	Quadratic term
Small	EF19	-8.943	0.076	1.2×10^{-3}
	EF28	-8.979	0.073	-0.2×10^{-3}
	EF49	-8.947	0.080	-0.7×10^{-3}
	EF50	-8.965	0.078	1.4×10^{-3}
	EF58	-8.939	0.078	0.4×10^{-3}
	EF63	-8.966	0.075	0.5×10^{-3}
	EF64	-8.979	0.074	0.1×10^{-3}
	EF7	-8.968	0.073	-0.4×10^{-3}
	EF86	-8.926	0.083	1.4×10^{-3}
	EF88	-8.976	0.079	-2.2×10^{-3}
Large	EF19	-7.223	0.053	3.5×10^{-3}
	EF28	-7.260	0.050	2.1×10^{-3}
	EF49	-7.227	0.058	1.5×10^{-3}
	EF50	-7.245	0.055	3.7×10^{-3}
	EF58	-7.220	0.056	2.6×10^{-3}
	EF63	-7.246	0.053	2.8×10^{-3}
	EF64	-7.260	0.052	2.4×10^{-3}
	EF7	-7.249	0.051	1.9×10^{-3}
	EF86	-7.207	0.061	3.6×10^{-3}
	EF88	-7.256	0.057	0.1×10^{-3}